PHYCOREMEDIATION OF NH_4^+ AND PO_4^{3-} FROM MEAT PROCESSING WASTEWATER BY USING MICROALGAE *BOTRYOCOCCUS* SP.

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DEDICATION

"To almighty God…."

To my beloved mother,

Biremavathi Sambasivam

whom I still miss everyday,

for being the backbone of the first part of my life

(I never had a chance to show her my love when she was alive)

To my fiancée,

Sharini Janasekaran

whom I can't afford to miss any day,

for being my backbone now and forever

(I'll never miss any chance to show her my love)

To my supervisor,

Assoc. Prof. Dr. Radin Maya Saphira Radin Mohamed

Who understands and guided me through the journey of my study.

For her patience, support and encouragement through all these years

And for believing in me and keep whispering

"You can do it Vicky"

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ABSTRACT

Phycoremediation is purposed to remove or biotransformation of pollutants including nutrients from wastewater. The excessive content of nutrients in a water body will lead to high concentration of undesirable microorganisms such as phytoplankton. This will lead to more serious problems that affects the ecosystem and brings human health problems. In over many years ago, considerable researches have been carried out on use of microalgae in wastewater treatment but the effect on particular type of wastewater discharged from meat processing industry is less reported. Wastewater disposed from meat processing industry has high content of NH_4^+ and PO_4^{3-}. Hence, this study is carried out to determine the effectiveness of microalgae *Botryococcus* sp. to remove nutrients NH_4^+ and PO_4^{3-} from wastewater from meat food processing industry and establishes the characteristic of this wastewater. Five concentrations of *Botryococcus* sp., $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ cell/ml were cultivated with three samples of wastewater which are sample obtained at two peak hours, 9.00 a.m. and 12.00 p.m. and composite sample of wastewater obtained hourly from 8.00 a.m.-5.00 p.m. (the factory working hour). The sample obtained at 9.00 a.m. contains highest BOD, COD, TSS, NH_4^+ and PO_4^{3-} compared to 12.00 p.m. and composite samples. For the removal of NH_4^+ and PO_4^{3-}, the best microalgae concentration is $1x10^6$ cell/ml in which removal efficiency of NH_4^+ and PO_4^{3-} achieved 89.74-99.03% and 92.39-99.93% respectively. Factors affecting the *Botryococcus* sp. growth are also studied and found that pH 7, N:P ratio 2:1 and initial *Botryococcus* sp. concentration of 10^6 as the optimized factors. Biokinetic coefficients of NH_4^+ removal by *Botryococcus* sp. were determined as reaction rate constant, k = 1.72 mg NH_4^+ mg^{-1}*chl a* d^{-1}, half saturated constant, K_m = 52.29 mg/L and yield coefficient Y_N = 0.027 mg *chl a* mg^{-1} NH_4^+. and for PO_4^{3-} removal, k = 1.13 mg PO_4^{3-} mg^{-1} *chl a* d^{-1}, Km = 44.45 mg/L and Y_P = 0.038 mg *chl a* mg^{-1} PO_4^{3-}. This study concludes that, *Botryococcus* sp. has high efficiency in removing NH_4^+ and PO_4^{3-} from meat processing wastewater and can be used for larger scaled treatment.

ABSTRAK

Phycoremediasi adalah untuk menghapuskan atau biotransformasi bahan pencemar termasuk nutrien daripada air sisa. Kandungan berlebihan nutrisi dalam saliran air akan menyebabkan kepekatan tinggi mikroorganisma seperti fitoplankton. Ini kemudiannya membawa kepada masalah yang lebih serius yang menjejaskan ekosistem dan membawa masalah kesihatan manusia. Banyak penyelidikan telah dijalankan ke atas penggunaan mikroalga dalam rawatan air sisa tetapi penyelidikan mengenai air sisa yang dilepaskan dari industri pemprosesan daging adalah kurang dilaporkan. Air sisa dilupuskan dari industri pemprosesan daging maengandungi kandungan tinggi NH_4^+ dan PO_4^{3-}. Oleh itu, kajian ini dijalankan untuk menentukan kecekapan mikroalga *Botryococcus* sp. untuk menghapuskan nutrien NH_4^+ dan PO_4^{3-} dari air sisa pemprosesan daging dan menganalisa ciri-ciri air sisa tersebut. Konsentrasi *Botryococcus* sp., $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ cell/ml telah dikultur dengan tiga sampel air sisa yang diperolehi pada tiga waktu berbeza. Sampel diperolehi pada jam 9.00 pagi mengandungi BOD, COD, TSS, NH_4^+ dan PO_4^{3-} tertinggi. Untuk penyingkiran NH_4^+ dan PO_4^{3-}, konsentrasi yang terbaik adalah konsentrasi $1x10^6$ sel/ml di mana kecekapan penyingkirannya$^-$ mencapai 89.74-99.03% (NH_4^+) dan 92.39-99.93% (PO_4^{3-}). Faktor yang mempengaruhi pertumbuhan *Botryococcus* sp. juga dikaji dan didapati bahawa pH 7, nisbah N:P, 2:1 dan konsentrasi awal *Botryococcus* sp., $1x10^6$ sebagai faktor -faktor yang optimum. Pekali biokinetic bagi penyingkiran NH_4^+ oleh *Botryococcus* sp. ditentukan sebagai kadar tindak balas yang berterusan, k = 1.72 mg NH_4^+ mg^{-1}*chl a* d^{-1}, separuh tepu berterusan, K_m = 52.29 mg/L dan pekali hasil Y_N = 0.027 mg *chl a* mg^{-1} NH_4^+ dan untuk penyingkiran PO_4^{3-} pula, didapati k = 1.13 mg PO_4^{3-} mg^{-1} *chl a* d^{-1}, K_m = 44.45 mg/L dan Y_P = 0.038 mg *chl a* mg^{-1} PO_4^{3-}. Kajian ini menyimpulkan bahawa, Botryococcus sp. mempunyai kecekapan yang tinggi dalam menghapuskan NH_4^+ dan PO_4^{3-} dari pemprosesan daging air sisa dan boleh digunakan untuk rawatan berskala lebih besar.

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LIST OF ABBREVIATIONS

TN	-	Total Nitrogen
NH_4^+	-	Ammonia Nitrogen
NO_3^-	-	Nitrate
TP	-	Total Phosphorus
PO_4^{3-}	-	Orthophosphate
N:P	-	Nitrogen: Phosphorous
Chl a	-	Chlorophyll *a*
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
TSS	-	Total Suspended Solids
CO_2	-	Carbon dioxide
Mg	-	Magnesium
Ca	-	Calcium
Fe	-	Iron
Cu	-	Copper
Zn	-	Zinc
Pb	-	Lead
Cd	-	Cadmium
Hg	-	Mercury
Mn	-	Manganese
Rpm	-	Rotations per minute
UTHM	-	Universiti Tun Hussein Onn Malaysia
APHA	-	American Public Health Association
DNA	-	Dioxoribonucleic Acid
RNA	-	Ribonucleic Acid

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Water is used in the production of many materials and finished products in the industries. The commercial meat based food industry generates huge quantities of wastewater. Discharge of untreated or partially treated wastewater from meat processing industry into the environment lead to increase of nutrients and organic compounds in the water body (Lecompte *et al*. 2015). In a study conducted in Romania, it was found that wastewater produced by meat processing factories had highest concentrations of nitrogen, 2743.6 mg/L and phosphate, 328.4 mg/L compared other wastewater from food operation in that country (Cristian, 2010). The high level of nutrients will cause eutrophication of water body through a phytoplankton bloom (Cai *et al*., 2013 and Cristian *et al*., 2010). This impairment of water quality greatly reduces the usability of the water for everyday purposes.

The treatment that suits for meat processing wastewater consists of various processes; it is either chemically or physically. The purpose of the treatment is to reduce the suspended solid which can clog water channels as they settle under gravity, biodegradable organics and thus reduction of Biochemical Oxygen Demand (BOD). Those biodegradable organics can serve as food for undesirable microorganisms which will combine these matters with oxygen to yield energy they need. Hence, the other organisms in the water body will be lack of oxygen and nutrients and eventually will lead to high concentrations of unwanted algae such as phytoplankton.

Several species of decomposers microorganisms applied in wastewater treatment process. Among them utilization of microalgae which called phycoremediation is the most common due to the high benefits related to their application which include production of bio-fuel, bio-products as secondary products as well as absence of toxic byproducts (Abdel-Raouf et al., 2012). Application of microalgae for wastewater treatment is something innovative. Several studies in literature has revealed potential of microalgae to reduce nutrients and remove heavy metals from different types of wastewater such as acidic wastewater by *Botryococcus* sp. (Areco et al., 2013), municipal wastewater by *Botryococcus* sp. and *S. obliquus* (Martinez et al, 2000; Orpez et al., 2009), agricultural wastewater by *Botryococcus* sp. (An et al., 2003), industrial wastewater by *Botryococcus* sp. and *Chlorella saccharophila* (Chinnasamy et al., 2010) and livestock wastewater by *Botryococcus* sp. and *Chlorella sp.* (Shen et al., 2008).

Some authors indicated that the phycoremediation by using of microalgae can be used for the treatment of raw wastewater, the results revealed high efficiency for the reduction of nutrients from domestic wastewater by using *Botryococcus* sp. (Rinna et al., 2014). However, the potential and biokinetics of these microalgae in the phycoremediation application of meat processing wastewater has not investigated extensively. This gap offered researchers a greater opportunity to explore the microalgae potential and its removal mechanism in the phycoremediation of these meat processing wastewater.

Theoretically, meat processing wastewater has high contents of nutrients necessary for microalgae growth (Fung *et al*., 2013). Moreover, this study aims to investigate the potential of *Botryococcus* sp. which was chosen based on its characteristics which make it more efficient in the phycoremediation as reported in literature.

1.2 Problem Statement

As the country develops, the problem of providing sufficient clean water to the population also grows. Until about fifty years ago, Malaysia's waste disposal system was no different from what is still found in many developing countries. As reported by Department of Statistics Malaysia (2012; 2013) percentage of clean rivers in Malaysia has declined significantly from 63.6% to 52.8% in five years from 2007 to 2012. In Malaysia, sufficient attention is not given to wastewater discharge of small industries like meat food processing industry.

The common process of wastewater treatment needed in meat processing factories, includes regulation, aeration, and settling tanks. Normally this mechanical aeration will be very expensive. Therefore, the small meat food processing industries which are basically has low production fails to treat the wastewater appropriately before discharging into drains and subsequently polluting the rivers. In this study, wastewater containing high phosphate and nitrogen is disposed from the small meat food processing industry in Parit Raja into a stream without necessary treatment. These nutrients can cause eutrophication of the water body and harms the ecosystem and brings human health problems. Hence, it is vital to carry out researches on the usage of microalgae to remove those nutrients from wastewater.

1.3 Objectives of Study

The study was undertaken to achieve the following objectives;

1. To characterise meat processing wastewater quality through BOD, COD, TSS, pH, TN, NH_4^+ and PO_4^{3-} concentrations.
2. To investigate the efficiency of microalgae *Botryococcus* sp. in removing NH_4^+ and PO_4^{3-} from meat processing wastewater during phycoremediation process.
3. To optimise the pH, N: P ratio and initial cell concentration of microalgae *Botryococcus* sp. to get the best growth rate of microalgae *Botryococcus* sp.
4. To evaluate the effects of nutrients removal efficiency through biokinetic coefficients, reaction rate constant (k), half saturated constant (K_m) and yield coefficient (Y) by using Michaelis–Menten rate expression.

1.4 Scope of Study

Botryococcus sp. is the microalgae selected to be used in this study. These microalgae is obtained from Microbiology Laboratory in University Tun Hussein Onn Malaysia

(UTHM) and originated from Endau Rompin National Park, Johor. Meat food processing industry wastewater was collected in a meat food processing small industry at Parit Raja. The quality of wastewater was evaluated based on characteristics including biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solid (TSS), pH, ammonium (NH_4^+) and orthophosphate (PO_4^{3-}). The phycoremediation process was conducted in lab scale and parameters ammonium (NH_4^+) and orthophosphate (PO_4^{3-}) tested to determine the efficiency of phycoremediation. The optimized parameters pH and N:P ratio and initial cell concentration of *Botryococcus* sp. was found by counting the cell concentration every day. The biokinetics of microalgae in phycoremediation of meat processing wastewater was studied using Michaelis–Menten rate expression model by determining the coefficients k, K_m and Y.

1.5 Hypothesis of the Study

This research hypothesized the microalgae *Botryococcus* sp. is potential to remove the nutrients, NH_4^+ and PO_4^{3-} from meat processing wastewater through phycoremediation. It is also expected that the growth of microalgae *Botryococcus* sp. is also influenced by pH and N:P ratio of the media and the initial concentration of the microalgae itself. The biokinetics coefficients k, K_m and Y of phycoremediation of meat processing wastewater studied using Michaelis–Menten rate expression model is expected to reveal the capability of *Botryococcus* sp. to remove high quantity of NH_4^+ and PO_4^{3-}.

1.6 Significance of Study

The primordial purpose of this study is to provide wastewater treatment enthusiasts with a complete and balanced education on wastewater treatment using microalgae as much as possible. Thus, this study will serve as the basis for future plans of action. Furthermore, this study will serve as a theoretical model for future studies of the same nature. Future researchers will benefit from this study, and it will provide them the facts needed to compare their study during their respective time and usability. Since only the Environmental Quality (Sewage and Industry Effluences) 1979 are used to apply the discharges of effluences, this study would assist Malaysian water resource authorities for the treatment of meat processing wastewater.

Besides that, this study which focused on microalgae *Botryococcus* sp. to remove the nutrients from meat processing wastewater is natural where microalga is an excellent source of environmental friendly. The nutrients are not being wasted while being consumed by the microalgae and eventually turned into valuable biomass product. In addition, microalgae provide a productive way to consume the nutrients and at the same time providing required oxygen for the aerobic bacteria through photosynthesis process.

Algae based wastewater treatment is said to be more cost saving method compared to any other secondary treatment method of wastewater treatment. This method is sustainable too as much consideration given to sustainable practices due to the need and taking into consideration of present concept among engineering society. This is in line with the definition of sustainable practices, where this defined as living life in a way that uses resources in a responsible way.

1.7 Structure of the Thesis

In Chapter 1, introduction mainly concentrated on the introduction to the background of study, problem statements, aim and objectives, scope of study, expected outcomes and the significance of study. While, in Chapter 2, literature review focuses on critical literature review on previous research. It is directly related to the thesis, providing information on theories, models, materials and technique. In Chapter 3, methodology, explains on the method used in investigating and determining the use of microalgae to remove the nutrients. This is summarized by the flow chart which clearly shows the flow adopted in completing this research.

Chapter 4, data analysis and results, explains the data analysis and results obtained whether the research carried out achieved the objectives set. The results obtained are discussed based on concrete evidences. Finally, Chapter 5 which includes conclusion and recommendations, focuses on conclusion of research carried out where it explains on the objectives, the achievements and suggestion for future improvement on research carried out.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter describes overview of related theory and previous research relevant to the study of removal of nutrients Orthophosphate (PO_4^{3-}) and Ammonium (NH_4^+) from meat processing wastewater using the microalgae, *Botryococcus* sp. The characteristics of wastewater from meat food processing industry and the microalgae, *Botryococcus* sp. will be discussed in this chapter.

2.2 Meat Food Processing Industry

Meat food processing industry in Malaysia mainly processes poultry meat (chicken), bovine meat (cattle), ovine meat (goat and sheep) and seafood (fish, prawn and crab) (Pazim *et al*., 2009). This meat food processing industry normally manufactures, meat based products such as sausages, meatballs, burgers, pickled meat, canned meat, dried, curried or spiced meat, meat floss, meatloaves and minced meat. There are 32 main companies involved in food processing in Malaysia and more than 50 small scale entrepreneurs actively involved (Ghani *et al*. 2010).

The commercial meat based food industry generates huge quantities of solid wastes and wastewater. The solid waste mainly composed of the whole waste animals which is rejected or unsatisfactory, carcasses, skeleton and animal waste, offal containing viscera and skin mostly produced from the evisceration and cleaning process of those animals (Owens, 2010). The wastewater mainly consists of the organic contaminant such as blood, animal feces, fats, oils and greases, fine parts of eviscerated organs and flesh and other parts in form of soluble, colloidal and particulate which are mostly produced from cleaning, washing and cutting of raw materials. This wastewater is supposed to be treated before discharged to the drainage (Indu *et al*. 2006; Owens, 2010).

2.2.1 Environmental Effects from Untreated Wastewater Disposal

Wastewaters have high organic and nutrients loads which included nitrogen, phosphorous and elements (Fe, Mn, Cu, Zn, Pb, Cd and Hg), as well as high oils and grease, salt, and pathogens (*Salmonella* sp. and *Campylobacter* sp.) (Cai *et al.*, 2013; Tritt *et al.*, 1992 and Sunda *et al*., 1998). Excessive nutrients and elements in the wastewater can cause eutrophication and disrupt the balance of the ecosystem. The discharge of untreated wastewater into the drainage develops the high nutrient and elements loading into aquatic environment of the drainage, which lead to a favourable condition for the water bloom of the undesired phytoplankton (Cai *et al*., 2013).

Phytoplanktons are organisms which is competent of absorbing the compulsory substances for growth and reproduction exactly from the surrounding water (Kuroshi, 2012 & Sunda *et al*. 1998). The two most vital nutrients needed for the growth of phytoplankton are nitrogen and phosphorous which are in vast amount in the meat processing wastewater discharged and in favourable Malaysian whether, it will be convenient for the phytoplankton bloom to take place (Cai *et al*. 2013; Nursuhayati *et al*. 2013; Anderson *et al*. 2002).

When the phytoplankton bloom takes place, this phytoplankton turns dangerous. It may cause hypoxia, dissolved oxygen (DO) depletion as the large number of dead phytoplankton decay as large amount of dissolved oxygen is used in the decaying process and directly causes 'fish kills' due to anoxia (Hallegraef, 2010; White *et al*. 1981). Depletion of dissolved oxygen occurs more at night as the phytoplankton use more oxygen than they give off during the day in photosynthesis process. As it causes 'fish kills', it eventually disorders the biodiversity of the ecosystem (Kangur *et al*. 2013; De Silva, 2012).

Besides that, some phytoplankton produces toxic chemicals when they die that can bring hazard other aquatic organisms. (Anderson *et al*. 2002). Toxicity can sometimes cause serious illness and even death to animals that consume the bio-toxin containing water (Berdalet *et al*. 2016). When humans subjected to this toxin they might suffer severe signs such as memory loss, diarrhea, gastroenteritis and lung irritations, paralysis and in serious case causes death (Hallegraef, 2003). The toxins can alter zooplankton communities, decrease growth of trout, and obstruct with growth of fish and amphibians. Therefore, it is obvious that the phytoplankton bloom caused by the meat processing industry can cause decline or loss of species biodiversity in the water body (Berdalet *et al*. 2016; Cai *et al*. 2013; Anderson *et al*. 2002).

Phytoplankton blooms cause the water body to have a bad odour, colour and taste. In addition, phytoplankton blooms produce isoprene, the second most abundant biologically derived greenhouse gas hydrocarbon will react with ozone and produces smog and emit methane when decay (Stephanie *et al*., 2010 & Sanderson *et al*., 2003). Smog particles may cause respiratory problems and cardiac diseases and damaging to the environment (Seaton *et al*. 2009). By causing phytoplankton bloom, untreated wastewater such as meat processing wastewater plays direct and indirect roles in causing air pollution and global warming (Paerl *et al*., 2012).

Other than the effect of the nutrients from the untreated wastewater from meat processing industry, there are some other effects to the water from this untreated disposal. The pollution potential of meat-processing is due to dissolved pollutants like blood from meat processing wastewater which have a chemical oxygen demand (COD) of 375 000 mg/L (Tritt *et al.*, 1992). Wastewater from meat food industry also contains excessive concentrations of suspended solids (SS), which includes hair, feathers, grease, flesh, pieces of fat, grit, manure, and undigested feed. 50% of the pollution charge is represented by these insoluble and slowly biodegradable SS while another 25% originated from colloidal solids (Sayed 1988). By producing high amount of pollutant, meat processing wastewater precipitates water pollution, and indirectly plays role in environmental defects rooted by water pollution (Bustillo-Lecompte *et al*, 2015).

2.2.2 Characteristic of Untreated Wastewater from Meat Related Activities

It can be noted that the wastewater from meat related activities mostly from slaughterhouse and meat processing factories is normally assessed in terms of mass parameters due to the specific amounts of wastewater and pollutant loads parallel to the animals slaughtered or processed that differ among the meat processing industry. Normally it contains significant amounts of total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC), chemical oxygen demand (COD), total suspended solids (TSS), and biochemical oxygen demand (BOD) (Tritt *et al*. 1992; Johns, 1995; Mittal, 2006; Cao *et al*. 2011; Wu *et al*. 2011; Barrera *et al*. 2012; Bustillo-Lecompte *et al*. 2013, 2014). Wastewater from meat related activities is regarded harmful worldwide because of its complex composition (Bustillo-Lecompte *et al.*, 2014).

The concentrations of nitrogen and phosphorous content in meat processing wastewater in previous studies shown in Table 2.2. The highest content of nitrogen and phosphorous among the meat processing wastewater is from meat processing wastewater with 2743.6 mg/L of nitrogen and 328.4 mg/L of phosphorous from the study by Cristian *et al*., (2010). This followed by slaughterhouse wastewater with 1057 mg/L of TKN and 217 mg/L of phosphorous from the study by Cassidy *et al*. (2005). The lowest amount of nitrogen and phosphorous is also found in the slaughterhouse wastewater with 51 mg/L. of N-NH^{-3} and 17.5 mg/L of phosphorous in the study by Akan *et al*. (2010).

Table 2. 1: Nitrogen and phosphorous content in wastewater from meat related activity from previous studies

Source of Wastewater	Nitrogen (mg/l)	Phosphorous (mg/l)	References
Meat processing	2743.6	328.4	Cristian, (2010)
Meat processing	199.5	51	Lecompte *et al*., (2015)
Slaughterhouse	700 (NH_4^+)	200 (PO_4^{3-})	Ge *et al*., (2013)
Slaughterhouse	1057 (TKN)	217	Cassidy *et al*., (2005)
Slaughterhouse	325	43	Jensen *et al*., (2014)
Slaughterhouse and meat packing	465 (NH_4^+)	176.5	Rajeshwari *et al*., (2000)
Slaughterhouse	51 (N-NH^{-3})	17.5	Akan *et al*., (2010)
Meat processing	91	29	Lu *et al*., (2015)
Slaughterhouse	190	50	Keller *et al*., (1997)
Meat processing	145 (TKN)	34	Nagalingam *et al*., (2002)
Poultry slaughterhouse	116 (N-NH^{-3})	57	Zheng *et al*., (2013)

In one of the studies from Table 2.1, which is conducted in Romania by Cristian, (2010), The results revealed that the wastewater produced by meat processing factories contains high amount of COD of 1683.6 mg/L, BOD of 863.4 mg/L, TSS of 640.2 mg/L and chlorides of 382.6 mg/L which are comparatively lesser than wastewater from milk and dairy products factory in that country with COD of 10251.2 mg/L, BOD of 4840.6 mg/L, TSS of 5802.6 mg/L and chlorides of 616 mg/L. However, it was found that meat factory wastewater contained highest concentrations of nutrients, nitrogen 2743.6 mg/L and phosphorous 328.4 mg/L with the N:P ratio of 8:1 as shown clearly in Figure 2.1 and Figure 2.2 (Cristian, 2010).

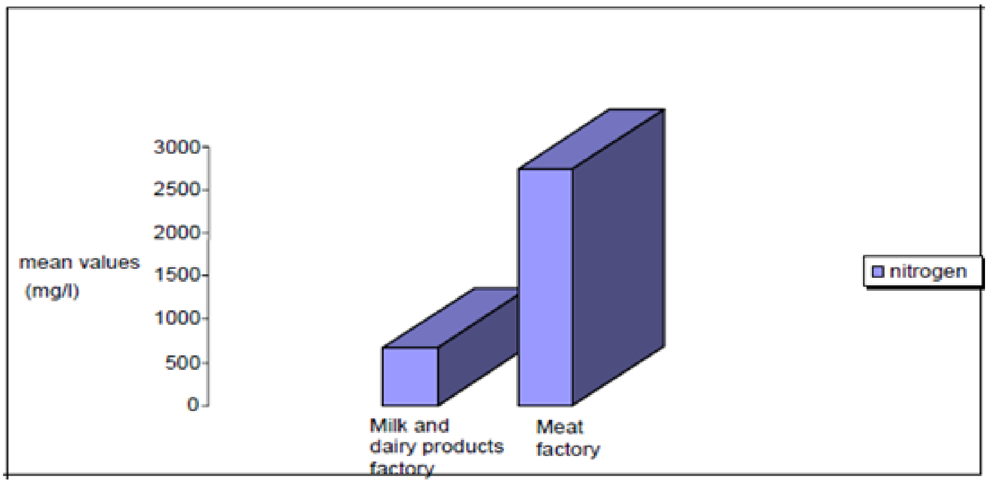

Figure 2.1: Comparative analysis of average values of nitrogen concentration (mg/l) determined in wastewater collected before treatment of the wastewater treatment supply units. (Cristian, 2010)

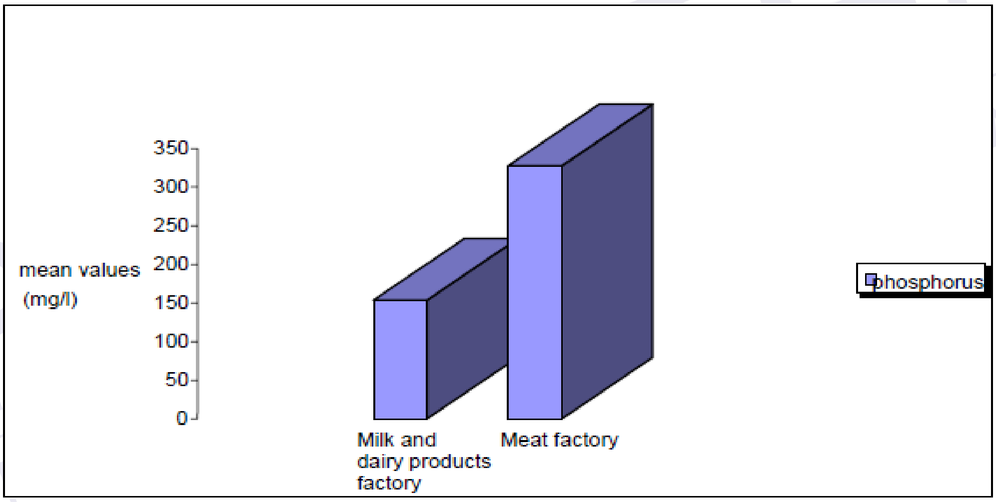

Figure 2.2: Comparative analysis of average values of phosphorus concentration (mg/l) determined in wastewater collected before treatment of the wastewater treatment supply units. (Cristian, 2010)

Lecompte *et al*. (2015) mentioned that 51% of the meat processing plants do not treat their meat processing wastewater onsite, 17% use aerobic treatment, 32% utilize passive systems such as storage tanks to settle solids, and only 2% utilize grease trap for fat separation and blood collection. The data of nutrients, nitrogen 60-339 mg/L and phosphorous 25.7-75.9mg/L with the N:P ratio of 2:1 to 4:1 is taken from selected provincially licensed plants from the survey.

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